ZO503 ANIMAL PHYSIOLOGY AND PHYSIOLOGICAL CHEMISTRY

BLOCK – III: PHYSIOLOGICAL CHEMISTRY

Enzymes

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ENZYMES

CONTENTS

13.1 OBJECTIVES

- To understand what are enzymes.
- To understand basic concepts, classification and their properties.
- Details of their mechanism of action and factors affecting enzyme activity
- To know about their sources, significance and deficiencies of enzymes.

13.2 INTRODUCTION

The life depends on a sequence of chemical reactions. The greatest majority of these biochemical reactions do not take place spontaneously. Most of the chemical reactions proceed too slowly on their own to sustain life. Hence catalysts are required to greatly accelerate the rates of these chemical reactions. Catalysis is defined as the “acceleration of a chemical reaction by some substance which itself undergoes no permanent chemical change”. The phenomenon of catalysis makes possible biochemical reactions necessary for all life processes. The catalysts of biochemical reactions are enzymes and are responsible for bringing about almost all of the chemical reactions in living organisms.

The existence of enzymes has been known for well over a century. French chemist Anselme Payen was the first to discover an enzyme, diastase, in 1833. A few decades later Louis Pasteur recognized in 1860 that enzymes were essential to fermentation but assumed that their catalytic action was intricately linked with the structure and life of the yeast cell. In 1877, German physiologist Wilhelm Kuhne (1837–1900) first used the term *enzyme*, which comes from Greek "leavened" (Leavening makes bread rise), to describe this process. Not until 1897 was it shown by German chemist Edward Buchner that cell-free extracts of yeast could ferment sugars to alcohol and carbon dioxide; Buchner denoted his preparation *zymase.* This important achievement was the first indication that enzymes could function independently of the cell.

The first enzyme molecule to be isolated in pure crystalline form was urease, prepared from the jack bean in 1926 by American biochemist J. B. Sumner, who suggested, contrary to prevailing opinion, that the molecule was a protein. In the period from 1930 to 1936, pepsin, chymotrypsin, and trypsin were successfully crystallized; it was confirmed that the crystals were protein, and the protein nature of enzymes was thereby firmly established.

13.3 DEFINITION

Enzymes are macromolecular biological organic catalysts responsible for supporting almost all of the chemical reactions that maintain animal homeostasis proceeds without itself being altered in the process.

13.4 BASIC CONCEPTS/GENERAL PROPERTIES

1. Enzymes differ from ordinary chemical catalysts by:
   - Higher reaction rates, 10^6-10^{12}
   - Milder reaction conditions (temp, pH)
   - Greater reaction specificity (no side products) - Capacity for regulation
2. Enzyme reactions are always reversible: they accelerate, or catalyze, chemical reactions.
3. Enzymes speed up the rates at which the equilibrium positions of reversible reactions are attained.
4. In terms of thermodynamics, enzymes reduce the activation energies of reactions, enabling them to occur much more readily
5. The reactants at the beginning of the process upon which enzymes may act are called substrates and the enzyme converts these into different molecules, called products.
6. Almost all metabolic processes in the cell need enzymes in order to occur at rates fast enough to sustain life.
7. The set of enzymes made in a cell determines which metabolic pathways occur in that cell.
8. The study of enzymes is called *enzymology*.
9. Enzymes are known to catalyze more than 5,000 biochemical reaction types.
10. Most enzymes are proteins, although a few are catalytic RNA molecules.
11. Enzymes' specificity comes from their unique three-dimensional structures, each enzyme catalyzes the reaction of a single type of molecules or a group of closely related molecules.

CHEMICAL NATURE AND STRUCTURE

All enzymes were once thought to be proteins, but since the 1980s the catalytic ability of certain nucleic acids, called ribozymes (or catalytic RNAs), has been demonstrated, contesting this saying.

A large protein enzyme molecule is composed of one or more amino acid chains linked together by peptide bonds. The amino acid sequence determines the characteristic folding patterns of the protein's structure, which is essential to enzyme specificity. If the enzyme is subjected to changes, such as variations in temperature or pH, the protein structure may lose its integrity (denature) and its enzymatic ability. Denaturation is sometimes, but not always, reversible. The key to enzyme activity is a structure called active site. Interactions between residues of polypeptide chain amino acids cause them to create a structure of defined size, shape and sequence. The active site is the region of an enzyme where substrate molecules bind and undergo a chemical reaction. The active site consists of residues that form temporary bonds with the substrate (binding site) and residues that catalyse a reaction of that substrate (catalytic site). The active site is usually a groove or pocket of the enzyme which can be located in a deep tunnel within the enzyme, or between the interfaces of multimeric enzymes.

Moreover, unrelated to its active site, there is an allosteric site on an enzyme, which can bind an effector molecule. This interaction is another mechanism of enzyme regulation. Allosteric modification usually happens in proteins with more than one subunit. Allosteric interactions are often present in metabolic pathways and are beneficial in that they allow one step of a reaction to regulate another step. They allow an enzyme to have a range of molecular interactions, other than the highly specific active site.

Bound to some enzymes is an additional chemical non-protein component called a cofactor, which is a direct participant in the catalytic event and thus is required for enzymatic activity. A cofactor may be either a coenzyme—an organic molecule (which is dialyzable, thermostable and loosely attached to the protein part), such as a vitamin—or an inorganic metal ion; some enzymes require both. A cofactor may be either tightly or loosely bound to the enzyme. If tightly connected, the cofactor is referred to as a prosthetic group (- an organic substance which is dialyzable and thermostable which is firmly attached to the protein or

apoenzyme portion.

This entire active complex is referred to as the holoenzyme; i.e., apoenzyme (protein portion) plus the cofactor (coenzyme, prosthetic group or metal-ion- activator) is called the holoenzyme.

Apoenzyme + Cofactor = Holoenzyme

ZYMOGEN: A zymogen is an enzymatically inactive precursor of an enzyme, often but not always a proteolytic enzyme (or proteinase). Some zymogens are named by adding the suffix -ogen to the name of the enzyme itself, as in trypsinogen or pepsinogen, whereas others are indicated by the prefix pro-, as in pro-collagenase or pro-carboxypeptidase.

13.5 NAMING AND ENZYME CLASSIFICATION

Except for some of the originally studied enzymes such as pepsin, rennin, and trypsin, most enzyme names end in "ase" to the name of their substrate describing their activity. Sometimes the same enzyme has two or more names, or two different enzymes have the same name. Because of such ambiguities, and the ever- increasing number of newly discovered enzymes, biochemists, by international agreement, have adopted a system for naming and classifying enzymes. This system divides enzymes into six classes, each with sub- classes, based on the type of reaction catalyzed. Each enzyme is assigned a four-part classification number and a systematic name, which identifies the reaction it catalyzes. The first Enzyme Commission, in its report in 1961, devised a system for classification of enzymes that also serves as a basis for assigning code numbers to them. These code numbers, proceed by EC (enzyme commission).

(i) The first number shows to which of the six main divisions (classes) the enzyme belongs,

(ii) The second figure indicates the subclass,

(iii) The third figure gives the sub-subclass,

(iv) The fourth figure is the serial number of the enzyme in its sub-subclass.

The main divisions are:

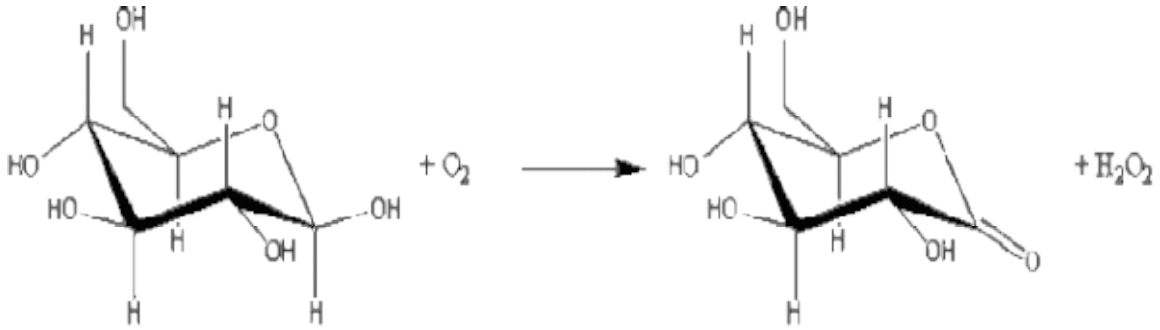
1. OXIDOREDUCTASES

Oxidoreductases are a class of enzymes that catalyze oxidoreduction reactions. Oxidoreductases catalyze the transfer of electrons from one molecule (the oxidant) to another molecule (the reductant). Oxidoreductases catalyze reactions similar to the following, $A^- + B \rightarrow A + B^-$ where A is the oxidant and B is the reductant. Trivial names of Oxidorecuctases include oxidases and dehydrogenases. Oxidases are enzymes involved when molecular oxygen acts as an acceptor of hydrogen or electrons. Whereas, dehydrogenases are enzymes that oxidize a substrate by transferring hydrogen to an acceptor that is either $NAD^+/NADP^+$ or a flavin enzyme. Other oxidoreductases include peroxidases, hydroxylases, oxygenases, and reductases.

An example, would be:

$$A^- + B \rightarrow A + B^-$$

where A=reductant (electron donor) and B=oxidant (electron acceptor).



b-D-glucose + oxygen ⟶ D-glucono-1,5-lactone + hydrogen peroxide

Hydroxylases add hydroxyl groups to its substrates. Oxygenases incorporate oxygen from Peroxidases are localized in peroxisomes, and catalyzes the reduction of hydrogen peroxide. molecular oxygen into organic substrates. Reductases catalyze reductions, in most cases reductases can act like an oxidases.

Oxidoreductase enzymes play an important role in both aerobic and anaerobic metabolism. They can be found in glycolysis, TCA cycle, oxidative phosphorylation, and in amino acid metabolism.

2. TRANSFERASES

Transferases are the class of the enzymes that catalyze the enact transfer of specific functional groups (e.g. a methyl or glycosyl group) from one molecule (called the donor) to another (called the acceptor). Transaminases, for example, catalyze the transfer of an amino group (−NH2) from an amino acid to an a-keto acid.[2] They are involved in hundreds of different biochemical pathways throughout biology, and are integral to some of life's most important processes.

Common names include acetyltransferase, methylase, protein kinase and polymerase. The first three subclasses play major roles in the regulation of cellular processes. The polymerase is essential for the synthesis of DNA and RNA.

Transferases are involved in innumerable reactions in the cell. For example, the activity of coenzyme A (CoA) transferase, which transfers thiol esters, the action of N-acetyltransferase is part of the pathway that metabolizes tryptophan, and also includes the regulation of pyruvate dehydrogenase (PDH), which converts pyruvate to acetyl CoA. Transferases are also utilized during translation. In this case, an amino acid chain is the functional group transferred by a peptidyltransferase. The transfer involves the removal of the growing amino acid chain from the tRNA molecule in the A-site of the ribosome and its subsequent addition to the amino acid attached to the tRNA in the P-site.

Based on the type of biochemical group transferred, transferases can be divided into ten categories (based on the EC Number classification). In the EC numbering system, transferases have been given a classification of EC2.

Systematically, a reaction would be:

$$X\,group + Y \xrightarrow{transferase} X + Y\,group$$

where, X= donor, and Y = acceptor. "Group" would be the functional group transferred as a result of transferase activity. The donor is often a coenzyme.

Example: Carbamyl phosphate + L-aspartate→ L-carbamyl aspartate + phosphate

3. HYDROLASES

Hydrolases are hydrolytic enzymes that catalyze the hydrolysis of a chemical bond, usually dividing a large molecule into two smaller molecules. Examples of common hydrolases include esterases, proteases, glycosidases, nucleosidases, and lipases.

Hydrolases carry out important degradativereactions in the body. During digestion, lipases hydrolyze lipids and proteases convert protein to amino acids. Hydrolases cleave large molecules into fragments used for synthesis, the excretion of waste materials, or as sources of carbon for the production of energy. In these reactions, many biopolymers are converted to monomers. Some hydrolases release energy as they act.

Systematically, a reaction would be:

$A–B + H_2O \rightarrow A–OH + B–H$

Example: Nucleases splits nucleic acids (DNA and RNA). Based on the substrate type, they are divided into RNase and DNase. RNase catalyzes the hydrolysis of RNA and DNase acts on DNA. They may also be divided into exonuclease and endonuclease. The exonuclease progressively splits off single nucleotides from one end of DNA or RNA. The endonuclease splits DNA or RNA at internal sites.

4. LYASES

Lyase is an enzyme that catalyzes the breaking (an "elimination" reaction) of various chemical bonds (C-C, C-O, C-N) by means other than hydrolysis (a "substitution" reaction) and oxidation. These bonds are cleaved by the process of elimination and the resulting product is the formation of a double bond or a new ring. The reverse reaction is also possible (called a "Michael addition"). For example, an enzyme that catalyzed this reaction would be a lyase:

$ATP \rightarrow cAMP + PP_i$

Lyases differ from other enzymes in that they require only one substrate for the reaction in one direction, but two substrates for the reverse reaction.

These bonds are cleaved by the process of elimination and the resulting product is the formation of a double bond or a new ring. This class of enzymes differs from other enzymes in that two substrates are involved in one reaction direction, but only one substrate is involved in the other direction. To generate either a double bond or a new ring, the enzyme is acted upon the single substrate and a molecule is eliminated.Lyases are classified as EC 4 in the EC number classification of enzymes

5. ISOMERASES

Isomerases are a class of enzymes which convert a molecule from one isomer to another, meaning that the end product has the same molecular formula but a different physical structure. Isomers themselves exist in many varieties but can generally be classified as structural isomers or stereoisomers. They can either facilitate it by intramolecular rearrangements in which bonds are broken and formed or they can catalyze conformational changes. The general form of such a reaction is as follows:

A–B → B–A

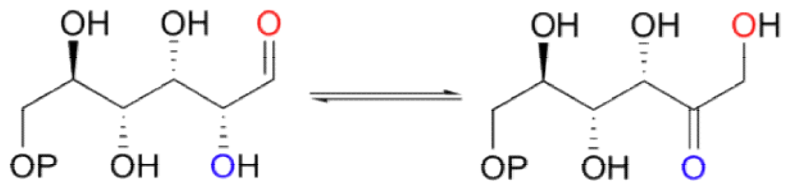
Where A and B = Isomers.

There is only one substrate yielding one product. This product has the same molecular formula as the substrate but differs in bond connectivity or spatial arrangements. Isomerases catalyze reactions across many biochemical pathways, such as in glycolysis and carbohydrate metabolism. All isomerases have Enzyme Commission numbers beginning in EC 5. A variety of isomerizations can be carried out, including racemization, cis-trans isomerization, enolization, and many others. Examples of isomerases include triose phosphate isomerase, and bisphosphoglycerate mutase.

Isomerases can help prepare a molecule for subsequent reactions such as oxidation-reduction reactions. Additionally, isomerases can catalyze phosphorylation reaction pathways throughout the Kreb Cycle by preparing the molecule for oxidation states. The change in

position is facilitated through Isomerases without affecting the overall chemical composition of the substrate or product.

Example:In the phosphoglucoseisomerase reaction, glucose-6-phosphate (an aldehyde sugar) and fructose-6-phosphate (a ketone sugar) are interconverted



glucose-6-phosphate fructose-6-phosphate

6. LIGASES

Ligases also called synthetases are the enzymes that catalyze reactions which make bonds to join together (ligate) smaller molecules to make larger ones. Ligase enzymes tend to raise the energy of a system, but the hydrolysis of ATP is often coupled with these reactions to make the reaction spontaneous. All enzymes tend to have the same basic catalytic effect in that they lower the overall activation energy often by moving the two substituents into close proximity. In general, a ligase catalyzes the following reaction:

$Ab + C \rightarrow A–C + b$

or sometimes

$Ab + cD \rightarrow A–D + b + c + d + e + f$

Where the lowercase letters denote the small, dependent groups. Ligase can join two complementary fragments of nucleic acid and repair single stranded breaks that arise in double stranded DNA during replication.

Example: a tyrosine-tRNA ligase is an enzyme that catalyzes the chemical reaction the 3 substrates of this enzyme are ATP, L-tyrosine, and RNA, whereas its 3 products are AMP, diphosphate, and L-tyrosyl-tRNA (Tyr).

This enzyme belongs to the family of ligases, to be specific those forming carbon-oxygen bonds in aminoacyl-tRNA and related compounds.

Tyrosine t-RNA Synthetase

ATP+ L-Tyrosine+ t-RNA ⟶ L-tyrosyl-tRNA + AMP + PPi

ENZYME UNITS

Enzyme activity is measured as the amount of the substrate lost per unit time.The enzyme commission of the International Union of Biochemistry (IUB) defined enzyme unit (U), later known as (IU), as the amount of enzyme that catalyzes the reaction of 1 μmol of substrate per minute under specified conditions.

Later the term "Katal" was introduced by the Commission on Biochemical Nomenclature as the SI (Systeme International) unit of enzyme activity as follow:The amount of enzyme causing loss of 1 mol substrate per second under specified conditions.

However, there is a direct relationship between the number of units of activity and the amount of sample assayed. Therefore to minimize these problems, units of enzyme activity may be related to the total protein content of the sample assayed, termed as "Specific activity", expressed as

International Units per mg protein or Katals per kg protein.

Turn-Over Number (k_{cat}): The number of substrate molecules converted into product in an enzyme-catalyzed reaction under saturating conditions in a given unit of time on a single enzyme molecule when the enzyme is saturated with substrate.

$k_{cat} = V_{max}/[E_{total}]$.

Where, $[E_{total}]$ = enzyme concentration and V max =maximum reaction rate

13.6 SPECIFICITY OF ENZYME ACTION

A characteristic feature of enzymes that makes them so important as diagnostic and research tools is the specificity they exhibit relation to the reactions they catalyze.some enzymes are specific for a particular type of chemical bond or functional group whereas few enzymes exhibit absolute specificity; that is, they will catalyze only one particular reaction. In general, there are four distinct types of specificity:

1. **Absolute, High or substrate specificity** – Such enzymes catalyze only one particular reaction.

Example:

a) Uricase, which acts only on uric acid.

b) Arginase, which acts only on arginine.

c) Carbonic anhydrase, which acts only on carbonic acid.

d) Lactase, which acts on lactose.

2. **Structural or Group specificity** - these enzymes act only on molecules that have specific functional groups, such as amino, phosphate and methyl groups.

Example:

a) Trypsin is an endopeptidase that hydrolyzes central peptide bonds in which the amino group belongs to basic amino acids e.g. arginine, lysine and histidine.

b) Chymotrypsin is an endopeptidase that hydrolyzes central peptide bonds in which the carboxyl group belongs to aromatic amino acids.

c) Aminopeptidaseis an exopeptidase that hydrolyzes peripheral peptide bond at the amino terminal (end) of polypeptide chain.

3. Linkage specificity–These enzyme acts on a particular type of chemical bond regardless of the rest of the molecular structure.

Example:

a) Amylase, which acts on α 1-4 glycosidic, bonds in starch, dextrin and glycogen.

b) Lipase that hydrolyzes ester bonds in different triglycerides

4. Stereo chemical specificity - these enzymes act on a particular steric or optical isomer.

Example:

a) L amino acid oxidase acts only on L amino acids. [L SEP]

b) D amino acid oxidase acts only on D amino acids. [L SEP]

c) α- glycosidase acts only on α- glycosidic bonds, which are present in starch, dextrin and glycogen. [L SEP]

ENZYME CATALYSIS

Enzyme catalysis is the increase in the rate of a chemical reaction by the active site of a protein. The mechanism of enzyme catalysis is similar in principle to other types of chemical catalysis.

An enzyme provides a specific environment within which a given reaction can occur more rapidly. An enzyme-catalyzed reaction takes place within the restricted pocket on the enzyme called the active site. The molecule that is bound in the active site and acted upon by the enzyme is called the substrate. The surface of the active site is lined with amino acid residues with substituent groups that bind the substrate and catalyze its chemical transformation. The enzyme-substrate complex, whose existence was first proposed by Charles-AdolpheWurtz in 1880, is fundamental to the action of enzymes.

MECHANISM OF CATALYSIS

A simple enzymatic reaction is as follows

E+ S ⇔ES ⇔EP ⇔E P

Where E=enzyme, S= substrate and P = product ES and EP are transient complexes of the enzyme with the substrate and with the product.

The function of a catalyst is to increase the rate of a reaction. Catalysts do not affect reaction equilibria. And so are the enzymes, the bidirectional arrows put in the equation on page make the point clear: any enzyme that catalyzes the reaction, S → P also catalyzes the reverse reaction, P → S. Its only role is to accelerate the interconversion of S and P. The enzyme is not consumed in the process, and the equilibrium point remains unaffected. However, the reaction reaches equilibrium much faster when the appropriate enzyme is present because the rate of the reaction is increased.

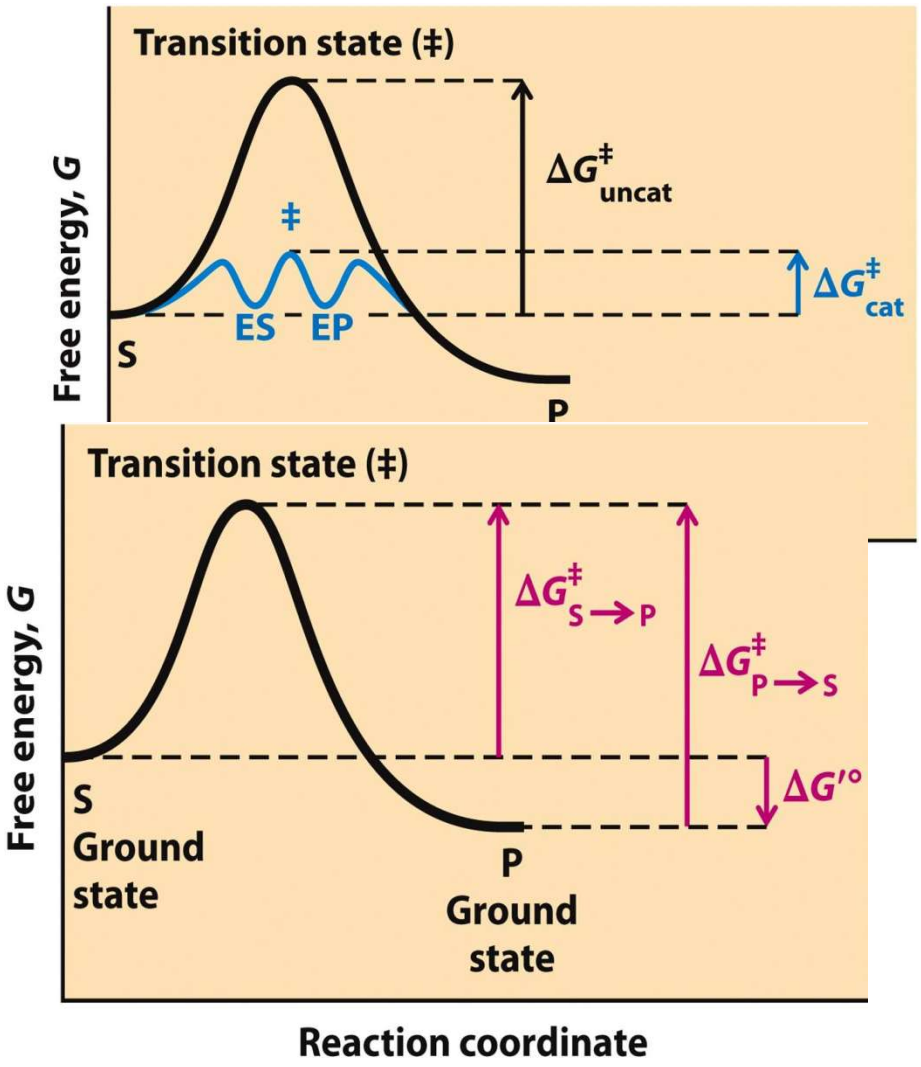
Energy in biological systems is described in terms of free energy, G. In the Fig no. 1-A, the free energy of the system is charted against the progress of the reaction (the reaction coordinate). The starting point for both the reaction (forward or reverse) is called the ground state, the contribution to the free energy of the system by an average molecule (S or P) under a given set of conditions. the free energy change for this reacting system under standard set of conditions (temperature, 298 K; partial pressure of gases, each 1 atm or 101.3 kPa ; pH = 0; concentration of solutes, each 1M) and call this as standard free-energy change, $\Delta G°$. Because biochemical systems commonly involve H+ concentrations far from 1M, biochemists define a constant $\Delta G'°$, the standard free-energy change at pH 7.0.

The equilibrium between S and P reflects the difference in the free energies of their ground states. the free energy of the ground state of P is lower than that of S, so G for the reaction is negative and the equilibrium favors P. The position and direction of equilibrium are not affected by any catalyst.

A favorable equilibrium does not mean that the S →P conversion will occur at a noticeable rate. There is an energy barrier between S and P: the energy required for arrangement of reacting groups, formation of transient unstable charges, bond rearrangements, and other alterations required for the reaction to proceed in both direction. To undergo reaction, the molecules must overcome this “energetic hill” or barrier and therefore must be raised to a higher energy level. At the top of the energy hill is a point at which deterioration to the S or P state is uniformly possible

This is called the transition state not be confused with a reaction intermediate (such as ES or EP). It is basically a transient molecular moment in which events such as bond breakage, bond formation, and charge development have proceeded to the precise point at which decay to either substrate or product is equally possible. The difference between the energy levels of the ground state and the transition state is the activation energy, G. The rate of a reaction reflects this activation energy: higher activation energy corresponds to a slower reaction. Reaction rates can be increased by raising the temperature, thereby increasing the number of molecules with sufficient energy to overcome the energy barrier. Alternatively, the activation energy can be lowered by adding a catalyst (Fig.no.1-B).Catalysts enhance reaction rates by lowering activation energies.

(A)



(B)

Figure No. 13.1 (A) Reaction coordinate diagram for a chemical reaction. (B)Reaction coordinate diagram comparing enzyme- catalyzed and uncatalyzed reactions

LOCK AND KEY MODEL

In order to explain why enzymes have such a high level of specificity, Emil Fischer in 1894 suggested that both a substrate and an enzyme have specific geometric shapes that fit exactly into each other **(Fig.No.2).** This enzyme-substrate complex is highly unstable and almost immediately this complex decomposes to produce the end products of the reaction and regenerates the free enzyme. The enzyme-substrate union results in the release of energy. It is this energy, which in fact, raises the energy level of the substrate molecule, thus inducing the *activated state*, in which certain bonds of the substrate molecule become more susceptible to cleavage.

This idea of both substrates and enzymes having a natural geometric fit has been called the lock and key hypothesis.

The problem with this hypothesis is that it doesn't explain the stabilization of the enzyme. When an enzyme has a substrate enter into its active site, the enzyme will change its shape slightly to match the substrate. If the enzymes were to be specifically designed to fit a substrate, then there would be no need for it to have to adjust its shape.

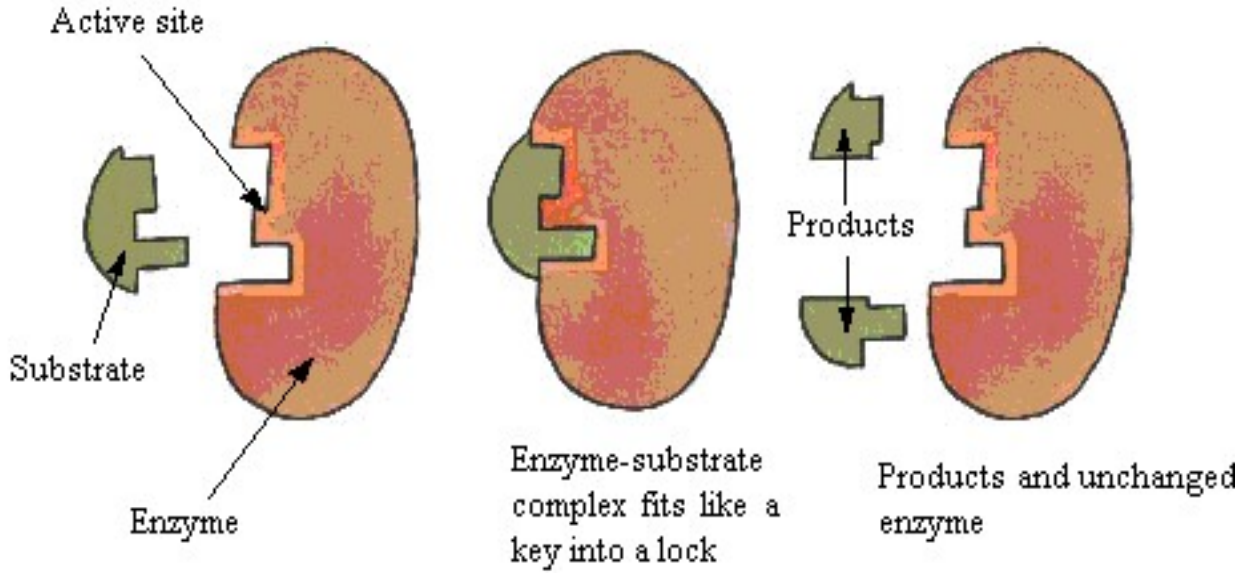


Fig. No. 13.2. Lock and Key model

INDUCED FIT MODEL

In 1958, another scientist named Daniel Koshland suggested a slight modification to the lock and key hypothesisto explain the enzyme properties more efficiently. Koshland's suggestion was that since enzymes were so flexible, the active site is constantly being reshaped by its interaction with the substrate. Koshland presumed that the enzyme molecule does not retain its original shape and structure. But the contact of the substrate *induces* some configurational or geometrical changes in the active site of the enzyme molecule (Fig No. 3). Consequently, the enzyme molecule is made to *fit* completely the configuration and active centres of the substrate.

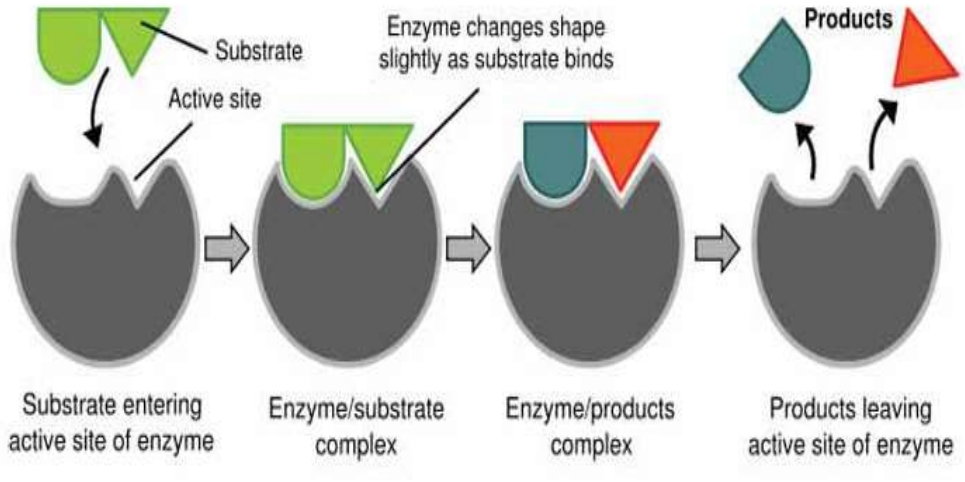


Fig.no. 13.3 Induced-fit Model

Moreover, that substrate doesn't bind to an active site as if it were specifically the right shape, but that the amino acid side-chains that are a part of the active site are molded into a specific position. This position allows the enzyme to start the catalyzing process. Koshland's modified suggestion has been called the induced fit theory.

Types of Catalytic Mechanisms

Enzyme catalyzed reactions are typically 10^7 to 10^{14} times faster than the uncatalyzed reaction.After binding takes place, one or more mechanisms of catalysis lowers the energy of the reaction's transition state, by providing an alternative chemical pathway for the reaction. There are seven possible mechanisms of "over the barrier" catalysis as well as a "through the barrier" mechanism:

1. Acid-base catalysis
2. Covalent catalysis
3. Metal ion catalysis
4. Proximity and orientation effects
5. Electrostatic catalysis
6. Bond strain
7. Quantum tunneling

These mechanisms are not mutually exclusive, and a given enzyme might incorporate several types in its overall mechanism of action. For most enzymes, it is challenging to compute the role of any one catalytic mechanism to the rate or specificity of a particular enzyme-catalyzed reaction.

1. Acid-Base Catalysis

The mechanism of acid- and base-catalyzed reactions is explained in terms of the Bronsted Lowry concept of acids and bases as one in which there is an initial transfer of protons from an acidic catalyst to the reactant or from the reactant to a basic catalyst. In terms of the Lewis theory of acids and bases, the reaction involves sharing of an electron pair donated by a base catalyst or accepted by an acid catalyst.

There are two types of acid-base catalysis:

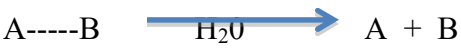
- General acid-base catalysis
- Specific acid-base catalysis

The term "general" refers to the fact that any acid or base we add to the solution will affect the rate of the reaction, and hence the catalysis is quite general. The term" specific" refers to the fact that just one acid or base, that from the solvent, affects the rate. The catalysis is therefore very specific.

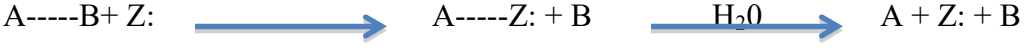
2. Covalent Catalysis

It involves a temporary covalent bond is formation between the enzyme (residues in the enzyme's active site or with a cofactor usually a nucleophile) and the substrate. This adds an additional covalent intermediate to the reaction, more reactive than the substrate itself originally was and helps to reduce the energy of later transition states of the reaction. The covalent bond must, at a later stage in the reaction, be broken to regenerate the enzyme. This mechanism is utilised by the enzymes such as proteases like chymotrypsin and trypsin.

Assume the hydrolysis of a bond between groups A and B:



covalent catalyst (an enzyme with a nucleophilic group Z:) the reaction becomes



This alters the pathway of the reaction, and it results in catalysis only when the new pathway has a lower activation energy than the uncatalyzed pathway. Both of the new steps must be faster

than the uncatalyzed reaction. The covalent bond formed between the enzyme and the substrate can activate a substrate for further reaction in a manner that is usually specific to the particular group or coenzyme.

3. Metal Ion Catalysis

Enzymes that bind metal ions tightly are referred to as metalloenzymes. Enzymes that bind metal ions more weakly, perhaps only during the catalytic cycle, are said to be metal activated. One role for metals in metal-activated enzymes and metalloenzymes is to act as electrophilic catalysts stabilizing the increased electron density that can develop during reactions.

The metal ion acts as a bridge between the substrate and the enzyme increasing the binding energy. Alternatively, the metal ion may bridge the substrate to a nucleophilic group. The metal ion may stabilize negative charges on a leaving group to make it a better leaving group, or shield negative charges on the molecule to allow for nucleophilic attack which otherwise may have been repelled. It may also participate in oxidation-reduction reactions by changing their oxidation state. An example is liver alcohol dehydrogenase where a zinc ion stabilizes negative charge development on the oxygen atom of acetaldehyde. Another potential function of metal ions is to provide a powerful nucleophile at neutral pH.

4. Proximity and orientation effects

Enzyme catalytic efficiency arises from the specific physical conditions at enzyme catalytic sites. Enzymes bring reacting species close together. It can accelerate a reaction between two species simply by holding the two reactants close together in an appropriate orientation. Enzymes, which have specific binding sites for particular reacting molecules, essentially take the reactants out of dilute solution and hold them close to each other. This proximity of reactants is said to raise the *effective* concentration over that of substrates in solution, and leads to an increased reaction rate. Enzymes not only bring substrates and catalytic groups together, they orient (specific geometric alignment) them in a manner suitable for catalysis as well. Clearly, proximity and orientation play a role in enzyme catalysis, but there is a problem with each for comparisons since we cannot separate true proximity and orientation effects from the effects of entropy loss when molecules are brought together.

By simply binding their substrates, enzymes facilitate their catalyzed reactions in three ways (+ electrostatic catalysis):

1. Enzymes bring substrates into contact with their catalytic groups and, in reactions with more than one substrate, with each other.

2. Enzymes bind their substrates in the proper orientations for reaction. Molecules are not equally reactive in all directions. Rather, they react most readily if they have the proper relative orientation.
3. Enzymes freeze out the relative translational and rotational motions of their substrates and catalytic groups.

In short this mechanism increases the rate of the reaction as enzyme- substrate interactions by aligning reactive chemical groups and holding them close together. This reduces the entropy of the reactants and thus makes reactions such as ligations or addition reactions more favorable, there is a reduction in the overall loss of entropy when two reactants become a single product.

5. Electrostatic catalysis

An electrostatic effect gives the largest contribution to catalysis. The enzyme provides an environment that is more polar than water, and the ionic transition states are stabilized by fixed dipoles. This is very different from transition state stabilization in water, where the water molecules must pay with "reorganization energy", in order to stabilize ionic and charged states. Thus, the catalysis is associated with the fact that the enzyme polar groups are preorganized

The magnitude of the electrostatic field exerted by an enzyme's active site is highly correlated with the enzyme's catalytic rate enhancement

Binding of substrate usually excludes water from the active site, thereby lowering the local dielectric constant to that of an organic solvent where electrostatic interactions are much stronger than they are in aqueous solutions. This strengthens the electrostatic interactions between the charged/polar substrates and the active sites. Furthermore, the charge distributions about the active sites are arranged so as to stabilize the transition states of the catalyzed reactions. In several enzymes, these charge distributions serve to guide polar substrates toward their binding sites so that the rates of these enzymatic reactions are greater than their apparent diffusion-controlled limits.

6 .Bond strain

This is the principal effect of induced fit binding, where the affinity of the enzyme to the transition state is greater than to the substrate itself. This induces structural rearrangements which strain substrate bonds into a position closer to the conformation of the transition state, so lowering the energy difference between the substrate and transition state and helping catalyze the reaction.

However, the strain effect is, in fact, a ground state destabilization effect, rather than transition state stabilization effect. Furthermore, enzymes are very flexible and they cannot apply large strain effect.In addition to bond strain in the substrate, bond strain may also be induced within the enzyme itself to activate residues in the active site.

5. Quantum tunneling

The "over the barrier" mechanisms above have been challenged in some cases by models and observations of "through the barrier" mechanisms (quantum tunneling). Some enzymes operate with kinetics which are faster than what would be predicted by the classical ΔG‡. In "through the barrier" models, a proton or an electron can tunnel through activation barriers. Quantum tunneling for protons has been observed in tryptamine oxidation by aromatic amine dehydrogenase. Interestingly, quantum tunneling does not appear to provide a major catalytic advantage, since the tunneling contributions are similar in the catalyzed and the uncatalyzed reactions in solution. However, the tunneling contribution (typically enhancing rate constants by a factor of ~1000 compared to the rate of reaction for the classical 'over the barrier' route) is likely crucial to the viability of biological organisms. This emphasizes the general importance of tunneling reactions in biology.

In 1971-1972 the first quantum-mechanical model of enzyme catalysis was formulated

13.7 FACTORS AFFECTING ENZYME ACTIVITY

The activity of an enzyme is affected by its environmental conditions. Changing these alter the rate of reaction caused by the enzyme. In nature, organisms adjust the conditions of their

enzymes to produce an optimum rate of reaction, where necessary, or they may have enzymes, which are adapted to function well in extreme conditions where they live.Several factors affect the rate at which enzymatic reactions - temperature, pH, enzyme concentration, substrate concentration, and the presence of any inhibitors or activators.

1. Temperature

Increasing temperature increases the kinetic energy that molecules possess, leading to more random collisions between molecules per unit time. Since enzymes catalyze reactions by randomly colliding with substrate molecules, increasing temperature increases the rate of reaction, forming more product.

As temperature increases more bond, especially the weaker ionic bonds will break as a result of this strain. As temperature increases, initially the rate of reaction will increase. However, the effect of bond breaking will become greater and greater, and the rate of reaction will begin to decrease.

The rate of enzyme activity increases with as temperature increases until the optimum temperature because of increased kinetic energy, then falls to zero as the enzyme is denatured. The temperature at which the maximum rate of reaction occurs is called the enzyme's optimum temperature. This is different for different enzymes. *Most enzymes in the human body have an Optimum Temperature of around 37.0 °C*

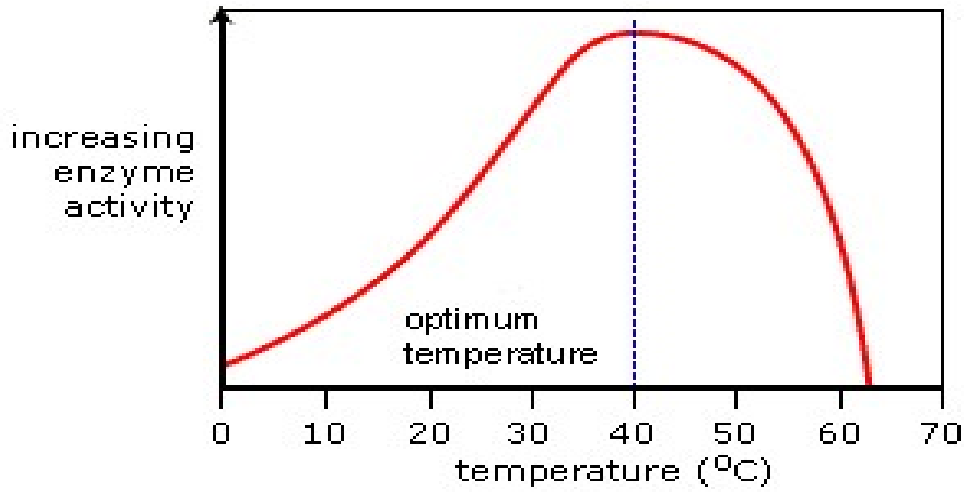


Fig. No. 13.4 Effect of Temperature on enzyme catalyzed reaction

2. pH - Acidity and Basicity

pH measures the acidity and basicity of a solution. It is a measure of the hydrogen ion (H^+) concentration, and therefore a good indicator of the Hydroxide Ion (OH^-) concentration. It ranges from pH1 to pH14. Lower pH values mean higher H^+ concentrations and lower OH^- concentrations. Acid solutions have pH values below 7, and Basic solutions (alkalis are bases) have pH values above 7. Deionised water is pH7, which is termed 'neutral'.

Each enzyme has its own range of pH in which it will work. H^+ and OH^- Ions are charged and therefore interfere with hydrogen and ionic bonds that hold together an enzyme, since they will be attracted or repelled by the charges created by the bonds. This interference causes a change in shape of the enzyme, and importantly, it's Active Site.

Different enzymes have different optimum pH values. This is the pH value at which the bonds within them are influenced by H^+ and OH^- Ions in such a way that the shape of their Active Site is the most Complementary to the shape of their Substrate. At the optimum pH, the rate of reaction is at an optimum. If pH increases or decreases much beyond this optimum, the ionisation of groups at the active site and on the substrate may change, effectively slowing or preventing the formation of the enzyme substrate complex. At extreme pH, the bonds which maintain the tertiary structure – hence the active site – are disrupted and the enzyme is irreversibly denatured. Any change in pH above or below the optimum will quickly cause a

decrease in the rate of reaction, since more of the enzyme molecules will have active sites whose shapes are not (or at least are less) complementary to the shape of their substrate.

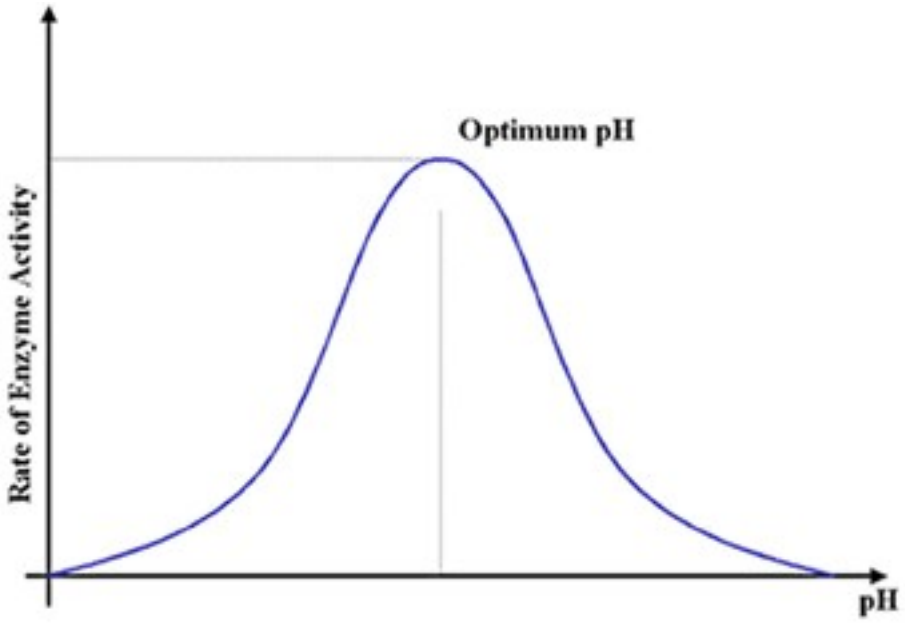


Fig. No. 13.5 Effect of pH on enzyme catalyzed reaction

Small changes in pH above or below the optimum do not cause a permanent change to the enzyme, since the bonds can be reformed. However, extreme changes in pH can cause enzymes to denature and permanently lose their function.

Enzymes in different locations have different optimum pH values since their environmental conditions may be different. *For example, the enzyme Pepsin functions best at around pH=2 and is found in the stomach, which contains Hydrochloric Acid (pH=2).*Most enzymes have an optimum pH that falls within the physiological range of 7.0-7.5.

3. Concentration

Changing the enzyme and substrate concentrations affect the rate of reaction of an enzyme-catalysed reaction. Controlling these factors in a cell is one way that an organism regulates its enzyme activity and so its metabolism.

A. Substrate Concentration

If we keep the concentration of the enzyme constant and increase the concentration of the substrate, it leads to increase in the rate of reaction. This is because more substrate molecules will be colliding with enzyme molecules, so more product will be formed.

However, after a certain concentration, any increase will have no effect on the rate of reaction, since substrate concentration will no longer be the limiting factor. The enzymes will effectively become saturated, and will be working at their maximum possible rate.

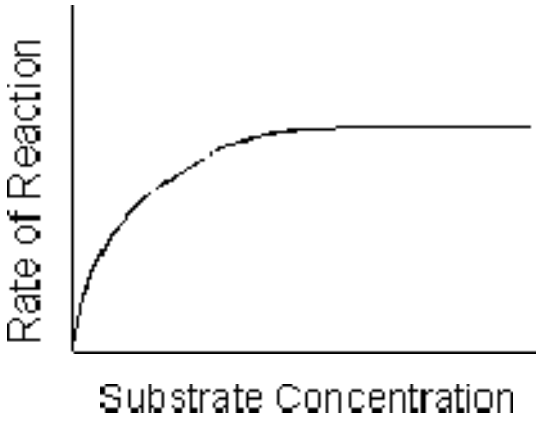


Fig. No. 13.6 Effect of varying substrate concentration on enzyme catalyzed reaction

B. Enzyme Concentration

If we keep the concentration of the substrate constant and increase the concentration of the enzyme, the rate of reaction increases linearly as more enzymes will be colliding with substrate molecules. Moreover, this is because rationally in all enzyme reactions the molar concentration of the enzyme is almost always lower than that of the substrate.

However, this too will only have an effect up to a certain concentration, where the enzyme concentration is no longer the limiting factor.

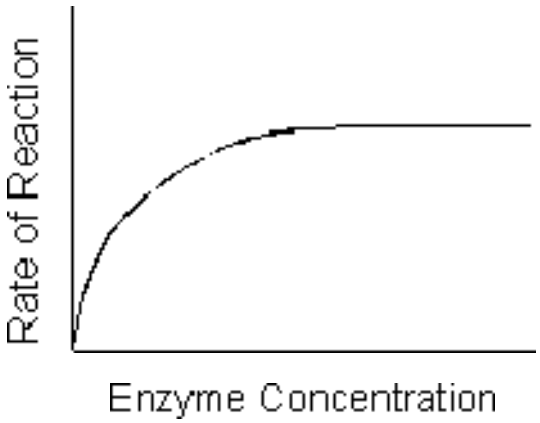


Fig. No. 13.7 Effect of varying enzyme concentration on enzyme catalyzed reaction

4. Cofactors

Many enzymes require cofactors to function properly. There are three main types of cofactor; co-enzymes, inorganic ions and prosthetic groups.

1. Coenzymes are organic molecules, which often contain a vitamin molecule as part of their structure. Coenzymes become loosely bound to the enzyme and move away from the enzyme once the reaction is completed. One coenzyme, e.g. NAD+ may react with many different enzymes in many different types of reaction. NAD+ transfers hydrogen in reactions involving dehydrogenase enzymes.

2. Inorganic metal ions are also known as enzyme activators. They change the charge in the active site, enabling the enzyme substrate complex to form. Some become intimately bound to the enzyme, e.g. Fe_2+ in catalase. Most others accelerate the binding between the enzyme and the substrate, e.g. Mg_2+ in phosphotransferases.

3. Prosthetic groups are coenzymes that bind permanently to the enzyme molecule and remain there even after the reactions are complete, e.g. FAD (flavin adenine dinucleotide). Like NAD+ it carries hydrogen atoms, this time with oxidase enzymes.

5. Inhibitors

Inhibitors slow down the rate of reaction. As such, they are an essential form of cellular control, allowing enzyme reaction rate to be slowed when necessary. Some enzymes are inhibited by the end product of the reaction they catalyse.

(a) Reversible inhibitors

There are two types of reversible inhibitor:

- Competitive reversible inhibitor
- Non-competitive reversible inhibitor

Competitive reversible inhibitors are structurally similar to the normal substrate and compete with the normal substrate for the active sites

However, if the concentration of the normal substrate is increased, reversible inhibitors are displaced from the active site and the normal enzyme substrate complex can form.

Non-competitive reversible inhibitors react with the enzyme but not at the active site. They change the shape of the whole enzyme, including the shape of the active site, hence the reaction cannot proceed and no products are formed on those enzymes

(b) Irreversible inhibitors

Irreversible inhibitors bind covalently and permanently to the enzyme, preventing normal enzyme function. For example, Aspirin is an irreversible inhibitor of cycloxygenase, an enzyme involved in the synthesis of prostaglandins. Substances such as mercury, iron and arsenic bind irreversibly to the SH (sulphydryl) group on enzymes.

13.8 SOURCES OF ENZYMES

Biologically active enzymes may be extracted from any living organism. A variety sources are used for commercial enzyme production. Of the hundred or so enzymes being used industrially, most of them are from fungi, yeast and bacteria with the rest divided between animal and plant sources. Microbes are preferred to plants and animals as sources of enzymes because of low production cost, enzyme contents are more predictable and controllable, easily arranged reliable supplies of raw material of constant composition, andplant and animal tissues comprise more potentially harmful materials than microbes, including phenolic compounds, endogenous enzyme inhibitors and proteases.Attempts are being made to overcome some of these difficulties by the use of animal and plant cell culture.

Enzymes from microbial sources:

Microorganisms are the most significant and convenient sources of commercial enzymes. They can be made to produce abundant quantities of enzymes under suitable growth conditions. Microorganisms can be cultivated by using inexpensive media and production can take place in a short period.

In addition, it is easy to manipulate microorganisms in genetic engineering techniques to increase the production of desired enzymes. Recovery, isolation and purification processes are easy with microbial enzymes than that with animal or plant sources. The great majority of microbial enzymes come from a very limited number of genera, of which *Aspergillus* species, *Bacillus* species and *Kluyveromyces* (also called *Saccharomyces*) species predominate. Most of the strains used have either been employed by the food industry for many years or have been derived from such strains by mutation and selection. For e.g. production of high fructose syrup using glucose isomerase and the use of pullulanase in starch hydrolysis.

Industrial production of enzymes aims at economy, effectiveness and safety and high yield as well. Among the microorganisms, *Aspergillus Niger* (a fungus) occupies a special position for the manufacture of a large number of enzymes in good quantities. There are well over 40 commercial enzymes that are conveniently produced by A. Niger. These include a-amylase, cellulase, protease, lipase, pectinase, phytase, catalase and insulinase.

A common trend in the industry today is that the gene coding for the enzyme with desired characteristics is transferred into one of the selected microbial production strains which have all the required features of safety and high expression levels and for which the growth medium has been optimised, hence avoiding the need for optimization of individual enzyme producing strains.

Enzymes from animal and plant sources:

Animal organs and tissues are very good sources for enzymes such as lipases, esterases and proteases. The enzyme lysozyme is mostly obtained from hen eggs. Some plants are excellent sources for certain enzymes-papain (papaya), bromelain (pineapple). Rennet has been among the most industrially significant enzymes obtained from animal tissue. The other enzymes obtained

from animal sources e.g. proteases like trypsin, chymotrypsin and urokinase, lactate dehydrogenase have diverse applications in industry, analysis,

In recent years, protein production in transgenic animals and –plants has attracted attention. Focus on transgenic animals (e.g. sheep, cattle) has been for the production of therapeutic proteins. The expression of the foreign gene is targeted to the mammary gland so that the protein is secreted directly into the milk.

Although both pharmaceutical and industrial proteins have been expressed in transgenic plants, they are suggested to be ideal bioreactors for production of the latter category of proteins. Production of bulk enzymes like α-amylase, xylanase, phytase, etc. combines the advantages of low production costs of plant biomass with the minimal purification requirements for such products.

Enzymes from mammalian cell cultures:

There exists a possibility of producing commercial enzymes directly by mammalian cell cultures. But the main constraint is the cost factor, which is extremely high. However, certain therapeutic enzymes such as tissue plasminogen activator are produced by cell cultures.

13.9 ENZYME DEFICIENCY

Causes of enzyme depletion

Unfortunately, enzymes are being depleted at every stage from seed to plate. This has caused enzyme deficiencies in the human body that leads to all kinds of health conditions. The main causes of enzyme depletion include:

- Pesticides and chemicals
- Hybridization and genetic engineering
- Bovine growth hormone
- Pasteurization
- Irradiated food
- Excess intake of unsaturated and hydrogenated fats

• Cooking at high temperatures

• Microwaving

• Radiation and electromagnetic fields

• Geopathic stress zones

• Fluoridated water

• Heavy metals

• Mercury amalgam dental fillings

Health disorders caused by enzyme deficiencies

Due to their critical role in a variety of functions in the body, enzyme deficiencies can cause many health related symptoms. The following are some of those health disorders associated with each of the four basic enzymes:

Protease (digests proteins): anxiety, low blood sugar, kidney problems, water retention, depressed immunity, bacterial and viral infections, cancer, appendicitis, bone problems (such as osteoporosis, arthritis, and bone spurs).

Amylase (digests non-fiber carbohydrates): skin problems such as rashes, hives, fungal infections, herpes, and canker sores; lung problems such as asthma, bronchitis, and emphysema; liver or gall bladder disease.

Lipase (digests fats): high cholesterol, obesity, diabetes, hardening of the arteries and other cardiovascular problems, chronic fatigue, spastic colon, dizziness.

Cellulase (digests fibers): gas and bloating, acute food allergies, facial pain or paralysis, candidiasis (bowel and vaginal yeast infections).

Beside these four types of deficiency, others are

Sucrase, Lactase & Maltase Deficiency

People who have malabsorption syndrome and cellulose deficiency also have a tendency towards sugar (sucrose, lactose, & maltose) and/or gluten intolerance. Sucrose, lactose and maltose are three common sugars which some people cannot tolerate. They are broken down and absorbed into the system by three enzymes; sucrase, lactase and maltase.

Sucrase deficient people cannot split the sucrose disaccharide into glucose and fructose. Glucose is a primary brain food so expect mental and emotional problems in people who are sucrase deficient. Symptoms include depression, moodiness, panic attacks, manic and schizophrenic behavior and severe mood swings.

People who are intolerant of lactose also have classic symptoms, which include abdominal cramps and diarrhea. Other allergic symptoms have been recorded, not the least of which was asthma, from the ingestion of lactose-containing products.

Maltase deficient people are generally sensitive to environmental conditions. An intolerance to sucrose, lactose or maltose may be worsened by a deficiency in sucrase, lactase or maltase.

Combination Deficiency

Combination deficiency is when an individual has more than one of the above deficiencies. The person will most often have the most severe digestive issues. Crohn's disease, Colitis, and Irritable Bowel Syndrome are quite common.

Gluten grains can be a real problem for example. These grains include wheat, oats, rye and barley. Not everyone has to avoid all four grains; however, sometimes it is a must. Gluten intolerance is associated with Celiac Disease and Malabsorption Syndrome. It is also associated with Crohn's Disease. Gluten is actually a protein that exists in these high carbohydrate grains. The best way to address this is usually a high potency protease and amylase enzyme combination.

The insidious thing about gluten intolerance is that it creates a sugar intolerance because when gluten intolerant people eat gluten containing foods, the brush border cells of the jejunum are injured and thus unable to secrete the disaccharidases (sucrase, lactase and maltase) leading to sugar intolerance. The problems discussed here are just the tip of the iceberg. More discoveries continue to emerge as research with food enzymes continues.

SIGNIFICANCE OF ENZYMES

Enzymes are needed for every chemical reaction that takes place in our body and are connected to every organ of the body. They are catalysts and are required by our body to digest food and ensure the delivery of vitamins and minerals. They work within the cells to regulate detoxification and produce energy. Enzymes can prevent partially digested proteins from putrefying, carbohydrates from fermenting, and fats from turning rancid within the body. They have been described as the 'live energy' of all organisms. Cooking, chemicals, and food processing destroy the natural enzymes found in the foods we eat and therefore, enzymes are by far the most important supplement to be taken. Enzymes from a plant-based source become active as soon as they enter the body whereas animal sources are only active within the small intestine in an alkaline setting.

The enzymes ensure the assimilation of vitamins, minerals, proteins, fats and carbohydrates and can help the body by supporting gall bladder function reducing inflammation, decreasing lactose intolerance, and aiding general indigestion.

THERAPEUTIC USES OF ENZYMES

Assay of plasma enzymes

Assay of plasma enzymes have been carried out routinely in clinical biochemistry whereas few of them have a clearly defined role in that particular location, the majority do not. For each of the plasma enzyme there is a normal concentration range or normal range of activity, which can be determined. Since many enzymes or isoenzymes are characteristically associated with the cells of certain tissues, their plasma assay can help to identify the location of damaged cells. This in turns correlate with the symptoms and case history of the patient and with other biochemical parameters. Examples are: Lactate Dehydrogenase (LDH), Alanine Transaminase (ALT), Alkaline Phosphatase (ALP), Aspartate Transaminase (AST), and Creatine Kinase (CK)

Inborn errors of metabolism

It forms a large class of genetic diseases involving congenital disorders of metabolism. The majority is due to defects of single genes that code for enzymes as a result of genetic mutation,

that facilitate conversion of various substances (substrates) into others (products). In most of the disorders, problems arise due to accumulation of substances, which are toxic or interfere with normal function, or to the effects of reduced ability to synthesize essential compounds.

The primary diagnosis is usually made by observing a build-up in plasma or urine of the metabolic intermediate, which is the substrate for the defective enzyme. There are many different types of inborn errors of metabolism.

A few of them are: Fructose intolerance, Galactosemia, Maple Sugar Urine Disease (MSUD), Phenylketonuria (PKU

ENZYMES AS REAGENTS IN CLINICAL BIOCHEMISTRY

Enzymes may be employed in a solution medium, immobilized on a surface of the reaction vessel the enzyme, as a catalyst, can be used as a label in various immunoassay techniques. Thus or in a reagent strip. The requirements imposed on the reagent enzyme may be different in all of these situations. For eg. D-glucose in blood and other physiological fluids is commonly analysed by involving glucose oxidase, this can be of use in diagnosis of diabetes mellitus. Blood lactate and pyruvate are usually determined by means of LDH-catalysed methods and blood urea is analysed by procedures involving urease. Beside this blood cholesterol may be analyse by using cholesterol oxidase. Interestingly, luciferase can be utilized that uses luciferin as a substrate to analyse the product oxyluciferin (exhibiting green chemiluminisence) on a spectrofluorimeter or luminometer

APPLICATION IN FORENSIC SCIENCE

A test for seminal acid phosphatase activity is used as a presumptive identification of semen. Detection of the presence of saliva in forensic samples can be detected by the presence of a-amylase, an enzyme that catalyses the hydrolysis of starch and glycogen. Alcohol dehydrogenase is used to monitor ethanol levels in forensic samples. RIA (Radioimmunosassay), ELISA (Enzyme linked immunosorbent assay) are being used to monitor the presence of agrochemicals and pharmaceuticals in biological samples. Assays relying on ELISA are also used to monitor serum proteins and for the post-mortem confirmations of HIV infections. Moreover several polymorphic enzymes can be used to establish individual's identity. Several other enzymes like

adenosine deaminase, adenylate kinase, carbonic anhydrase, glucose -6-phosphate dehydrogenase are important forensic markers.

Enzymes can be used for Aiding Digestion. Example: Amylases, Proteases and Lipase.They act as anti-clotting agents like Fibrinolytic and Thrombolytic. Examples: Urokinase and Streptokinase.Enzymes can be used as surface disinfectants. Example: Trypsin.

INDUSTRIAL PURPOSE

Enzymes can be used in the textile industry. Example: Amylase as softening agent for starched clothes.They can also be used for Leather purpose. Example: Proteolytic purpose.Enzymes has the importance in the paper manufacturing. Examples: Endoxylanases for bleaching of Wood pulp.They can be used in the manufacturing of organic compounds. Example: Bacterial enzymes for the manufacturing of acetone, butanol, lactic acid etc.

Enzymes as Food and in food industry:-

Enzymes can be used in the meat packing industry. Example: Papain which is proteolytic in action, therefore hydrolyses peptide bonds thus for tenderizing meat and beef. Enzymes have their role in Manufacturing of cheese. Example: Rennin (chymosin) found in stomach, converts milk protein casein to curd like calcium paracaseinate.Papain is used to stabilize chill proof bear. Yeast enzymes are also used in beverage industry. Lactose is used to prevent the formation of lactose crystals in ice-cream preparations.

Use in Baking

The wheat flour used for bread has naturally occurring enzymes that modify the starch, protein and fiber of the flour when water is added. Yeast added to the mixture also has enzymes, which ferment the maltose over time, to make the dough rise. In bakeries, the quality of the wheat flour varies, as a consequence of natural variation, time of year or inconsistencies in milling. To improve consistency and efficiency, extra enzymes (like xylanase, □-amylase, protease, glucose oxidase and lipase) are used as supplements, enabling better handling of the dough and the control of certain characteristics in the finished bread.

Use in Alcohol

In the alcohol industry, fermentation depends on the action of enzymes synthesised by the yeasts

and bacteria used in the production process. Beer brewing essentially involves the yeast action on barley, maize, sorghum, hops or rice. The yeast cells convert simple sugars into alcohol and carbon dioxide. However most sugar present is in the complex polysaccharide form such as starch and cannot readily be used. So these nutrients are "released" by malting in which enzymes are released, degrading starch and protein to simple reducing sugars and amino acids. The traditional malting process is an expensive inefficient way of manufacturing enzymes. So nowadays industrial enzymes such as amylases, glucanases and proteases are added to unmalted barley to produce the same products that malting would produce by more controlled means. Use of enzymes in the beverage industry allows it to be more economic and have consistent quality.

Use in Fruit Juices

Enzymes are used in the processing of fruit juices to maximize the production of clear or cloudy juice. Nearly all fruits contain pectin. The presence of soluble pectin in squeezed juice causes cloudiness. The addition of pectin degrading enzymes (pectin methyl esterase, polygalacturonase and pectin lyase) at the pressing stage increases the amount of juice produced and can reduce cloudiness. The desired flavour and colour of citrus juices especially orange depends on the insoluble, cloudy materials of the pressed juice. The pectin component is manipulated requiring a balance between pectin methyl esterase, to promote cloudiness by increasing the pectin/calcium complex formation and polygalacturonase, to break cloudiness by depolymerisation of the pectin. The application of enzymes in these processes is cosmetic.

Use in Washing Powders

Principally protease digests on organic stains such as grass, blood, egg and human sweat and lipases are effective on stains resulting from fatty products and amylases are effective on removing starchy food deposits. Some powders contain cellulase to brighten colours and soften fabrics. Protease and amylase are also effective in dishwasher detergents, to remove food particles. These detergents are environmentally friendly with fewer bleaching agents and phosphates, allowing the enzymes to do more work and have beneficial effects on public and environmental health.

Use in the Textile Industry

Enzymes are used in the leather and the textile industries in finishing processes. Proteases help in the de-hairing of the animal hides and lipases are used for de-greasing. The correct application of a cellulase enzyme can give a smoother, glossier brighter fabric to cellulose fibres like cotton. This technique is known as bio-polishing. In the denim industry, cloth was traditionally stonewashed with pumice stones to fade the fabric. A small application of cellulaseminimises damage to the garments and also to machinery. This technique is known as bio-stoning and can ensure greater fading without high abrasive damage to fabric and accessories (buttons, rivets). The use of enzymes in this area of industry illustrates their valuable technological contribution. Enzymes are also used in contact lense solution and in pet toothpaste.

Immobilized Enzymes

Immobilized enzymes are made by the attachment of an enzyme to an insoluble support which allows its reuse and continuous use and thus eliminating the tedious recovery process. Immobilization stabilizes the enzyme; moreover two or more enzymes catalyzing a series of reactions may be placed in close proximity to one another. Adsorption, covalent linkage, crosses linking, matrix entrapment or encapsulations are different methods for making immobilized enzymes. Production of glucose syrups from starch by the use of immobilized enzymes is one of the most important processes of food industry.

13.10 SUMMARY

- Enzymes are powerful and specific biological catalysts.
- They catalyze almost every biochemical reaction.
- All known enzymes are proteins with the exception of a few catalytic RNAs. Many require nonprotein coenzymes or cofactors for their catalytic function.
- Enzymes are classified according to the type of reaction they catalyze. All enzymes have formal E.C. numbers and names, and most have trivial names.
- The part of the Enzyme that acts a Catalyst is called the Active Site. The rest of the Enzyme is much larger and is involved in maintaining the specific shape of the Enzyme.
- When a reaction involving an Enzyme occurs, a Substrate is turned into a Product. The Substrate can be one or more molecules. The Active Site of an Enzyme is Complementary to the Substrate it catalyses.

- One of the properties of enzymes that make them so important is the specificity they exhibit relative to the reactions they catalyze.
- Enzymes increase the rate of a reaction by lowering its activation energy.

- The Lock-and-key Hypothesis is a model of how enzymes catalyse substrate reactions. It states that the shape of the active sites of enzymes areexactly complementary to the shape of the substrate.
- The recent model of Induced-Fit Hypothesis states that the shape of active sites is not exactly complementary, but change shape in the presence of a specific substrate to become complementary.
- Several factors affect the rate of enzymatic reactions - temperature, pH, enzyme concentration, substrate concentration, and the presence of any inhibitors or activators.
- They have enormous application in the field of medical science, forensics and industries.